

2004 Arctic Yukon Kuskokwim Sustainable Salmon Initiative Project Final Report¹

Effective population size of Chinook salmon in Yukon and Kuskokwim River
tributaries

by:

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Abstract

The effective population size per generation (N_e) is an important indicator of the genetic health and viability of a population. In this study, genetic and demographic data are used to estimate and evaluate contemporary N_e for Chinook salmon (*Oncorhynchus tshawytscha*) from the Gisasa and Tozitna rivers, tributaries of the Yukon River, and the Kwethluk and Tuluksak rivers, tributaries of the Kuskokwim River. The genetic estimates of N_e range from 2,307 to 7,674 when the populations are assumed to be isolated, but are much smaller when migration is assumed, ranging from 448 to 576. Estimates of the immigration fraction per generation (m) vary considerably, ranging from 0.07 to 0.09 for the Yukon River populations and 0.34 to 0.43 for the Kuskokwim River populations. The mean genetic estimate of the ratio N_e to census size (N) is 0.28, assuming isolation, and 0.04, assuming migration. The estimates of N_e/N vary widely among populations but clearly show that census size estimates are not accurate indicators of genetic health. A comparison of N_e/N ratios from genetic and demographic data suggests N_e is impacted most by unequal sex ratio and variance in reproductive success and least by fluctuating population size for the time period examined in this study. The results indicate the observed sex ratio bias could adversely impact the long term genetic health of these populations if annual run sizes, and migration rates, decline. Further, the results underscore the importance of maintaining population connectivity and suggest that the vulnerability to loss of genetic diversity varies regionally and is negatively correlated with gene flow.

Key words: Effective population size, Chinook salmon, Yukon River, Kuskokwim River, sex ratio bias.

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Introduction

Maintaining genetic diversity is necessary for maintaining healthy, viable populations. This tenet of conservation is most relevant for populations that are small or are experiencing significant declines in abundance. Small populations are of particular concern because loss of genetic diversity is inversely proportional to population abundance. In this context, abundance refers to the effective size (N_e) of the population, not the census size (N), and theory suggests genetic diversity is lost at a rate of $1/(2N_e)$ per generation (Frankham 1995). Thus, the effective population size is an important indicator of the genetic health and viability of a population. Conservation guidelines have been established from theoretical studies that suggest isolated populations having an N_e below 500 (50) are at risk of significant long-term (short-term) loss of genetic diversity (Lande and Barrowclough 1987; Waples 1990a; Allendorf et al. 1997). These threshold values of 500 and 50 provide a yardstick with which to evaluate N_e estimates.

Wright (1931) defined effective population size as the number of breeding adults in an “idealized population” that would lose genetic diversity at the rate observed in the actual population. This idealized population exhibits a constant size over generations, a 1:1 sex ratio, and random variation in individual reproductive success. Violations of these assumptions typically result in a reduction of N_e relative to N . For example, in a review of N_e and N in over 100 species, Frankham (1995) showed that the ratio N_e/N is, on average, about 0.11. Therefore a population with a census size of 250 may in fact be losing genetic diversity at a rate equivalent to that of an idealized population of about 28. In 39 generations this population would lose 50% of genetic diversity compared to only 8% for a population with N_e of 250. This disparity underscores the danger in relying solely on census population size to evaluate short and long term population health, especially when the census population size is small.

Both demographic and genetic data can be used to estimate N_e . Demographic data (estimates of census population size, sex ratio, variance in reproductive success) provide a direct estimate of N_e based on the concepts and analytical methods developed by Wright (1931, 1938). If sufficient demographic data exists, then multiple estimates of N_e can be derived, accounting for the effects of population size, sex ratio, and variance in reproductive success. In contrast, genetic data from neutral genetic markers provide an indirect estimate of N_e . These are comprehensive estimates in that they reflect the influence of all demographic variables. Genetic estimates of N_e are

frequently derived using the temporal method based on the concept that genetic diversity in a closed finite population decreases over time in inverse proportion to N_e . The temporal method for estimating N_e in Pacific salmon (*Oncorhynchus* spp.) is based on the analytical work of Waples (1990a, 1990b) and Tajima (1992). Comparing genetic and demographic estimates of N_e provides valuable insight into the relative influence of demographic variables (fluctuating population size, sex ratio, and variance in reproductive success) effecting N_e (e.g. Hansen et al. 2002; Palm et al. 2003; Ardren and Kapuscinski 2003; Shrimpton and Heath 2003).

Presently, there are no N_e estimates available for Chinook salmon (*O. tshawytscha*) populations in the Arctic/Yukon/Kuskokwim (AYK) region. Nevertheless, there is concern that demographic factors, including male-biased sex ratio and low escapement (spawning census size), may be reducing N_e to well below 500 in some populations. Four such populations are located in the Kwethluk and Tuluksak rivers from the Kuskokwim River drainage and the Gisasa and Tozitna rivers from the Yukon River drainage. Weirs operated intermittently between 1990 and 2003 indicate the percentage of adult female Chinook salmon averages 22% (Kwethluk River), 22% (Tuluksak River), 30% (Gisasa River), and 15% (Tozitna River). The annual escapement (N) averages 9,001 (Kwethluk River), 1,554 (Tuluksak River), 2,628 (Gisasa River), and 1,708 (Tozitna River). If we apply the average N_e/N ratio (0.134) derived by Shrimpton and Heath (2003) for five Fraser River Chinook salmon populations (similar in census size to the AYK populations), we obtain N_e estimates of 1,206, 208, 352, and 229 for the Kwethluk, Tuluksak, Gisasa, and Tozitna rivers. These point estimates must be viewed with caution because N_e/N ratios are quite variable. Nevertheless, three of the four values are well below 500, suggesting these populations may be at high risk of long-term loss of genetic diversity.

Objectives

This study has two objectives:

Objective 1. Use genetic and demographic data to estimate and evaluate the N_e of Chinook salmon in the Kwethluk, Tuluksak, Gisasa and Tozitna rivers.

- Four estimates of N_e were computed for each population. Two estimates of N_e were derived from genetic data. One estimate was made using the temporal method of Waples (1990a, 1990b) that assumes each population is isolated. A second estimate

was made using the likelihood method of Wang and Whitlock (2003) that assumes gene flow among populations and computes N_e and the immigration fraction (m) simultaneously. The method of Wang and Whitlock (2003) was not described in the project proposal because it is new and has only been used in recent studies of Pacific salmon (e.g. Ford et al. 2004). Nevertheless, migration likely occurs among these populations and estimates of N_e that account for gene flow may better reflect salmon in this region. Two estimates of N_e were made from demographic data using sex-specific census size (annual escapement) data from each population. We did not use the data on variance in reproductive success from a California Chinook salmon population (Hedrick et al. 2000) as described in the project proposal. This data was deemed inappropriate since it was derived from a hatchery population. As an alternative, the impact of variance in reproductive success on N_e was inferred by comparing the demographic and genetic estimates N_e .

Objective 2. Evaluate the relative influence of the demographic variables (spawning census size, sex ratio, and variation in reproductive success) on N_e .

- The genetic and demographic estimates of the ratio N_e/N were compared to infer the relative influence of fluctuating population size, sex-ratio bias, and variance in reproductive success on N_e . In addition, the two genetic estimates of N_e were compared to evaluate the likely role of migration (gene flow) on genetic diversity in each population.

Methods

Study Area

The Gisasa and Tozitna rivers are in the middle Yukon River drainage (Figure 1). The Gisasa River flows northeast approximately 112 km from its headwaters in the Nulato Hills to the Koyukuk River about 90 km upstream from the Yukon River. The Tozitna River flows southwest approximately 207 km from its headwaters in the Ray Mountains to its confluence with the Yukon River. The Gisasa and Tozitna River weirs are located about 4 km and 80 km upriver from their respective mouths.

The Kwethluk and Tuluksak rivers are tributaries to the lower Kuskokwim River. The Kwethluk River flows northwest approximately 222 km from its headwaters in the Eek and Crooked Mountains to its confluence with the Kuskokwim River. The Tuluksak River flows northwest approximately 137 km from its headwaters in the Kilbuck Mountains to its confluence with the Kuskokwim River. The Kwethluk and Tuluksak River weirs are located about 88 km and 49 km upstream from their respective mouths.

Detailed descriptions of the climate, habitat, and river characteristics can be found in the project reports summarizing Chinook salmon escapement and run timing (e.g. Wiswar 2001; Gates and Harper 2003; Roettiger et al. 2003)

Sample collection, preparation, and genotyping

Archived tissue samples were used as the source of DNA. Fin tissue samples from live adult Chinook salmon were collected at weirs in each of the four rivers between 2001 and 2003 (Figure 1, Table 1). Heart and fin tissue samples from juvenile Chinook salmon (Kwethluk River only) were collected in 1990 and 2003, respectively, using minnow traps. Fin tissue was stored in 2 ml vials and preserved in 100% ethanol. Heart tissue was stored in 2 ml vials and frozen at -70°F. Age-at-return data for each individual [K. Harper, U.S. Fish and Wildlife Service (USFWS) unpublished data; K. VanHatten, USFWS unpublished data; C. Kretsinger, Bureau of Land Management (BLM) unpublished data] was used to stratify adult samples by cohort for estimation of N_e from genetic data (Table 1). As suggested by Waples (1990b), only cohorts having a minimum sample size of 50 were used. The total population sample size ranged from 219 (Tuluksak River) to 516 (Kwethluk River).

Ten microsatellite loci were used to genotype each sample (*Oke2* and *Oke4*, Buchholz et al. 2001; *Ots3.1*, Banks et al. 1999; *Oki10* and *Oki11*, Smith et al. 1998; *Ots311*, *OtsG3*, *OtsG253b*, *OtsG432*, and *OtsG474*, Williamson et al. 2002). Total genomic DNA was isolated from approximately 10-20mg of fin tissue using the Qiagen 96-well Dneasy® procedure. Isolated DNA was quantified using a 96-well Packard FluoroCount® Microplate Fluorometer and diluted to 30ng/μl for use in PCR. PCR was conducted in 10 μl volumes consisting of 0.06 units of Taq polymerase, 1μl of 30ng DNA, 1.5-2.5mM MgCl₂, 1mM 10x buffer, 0.8mM dNTP's, 0.006-0.065μM of labeled forward primer (depending on locus), 0.4μM unlabeled forward primer, 0.4μM unlabeled reverse primer, deionized H₂O, and 1M Betaine (majority of loci). PCR was

completed on an MJResearch™ DNA Engine™ PCT-200 or a DNA Engine Tetrad™ PCT-225. The amplification profile consisted of one cycle of 2 min @ 92°C, 30 cycles of 15 sec @ 92°C, 15 sec @ 52-60°C (depending on locus) and 30 sec @ 72°C, and a final extension for 10 min @ 72°C. Microsatellites were separated on 64-well denaturing polyacrylamide gels utilizing Li-Cor IR² scanners and Li-Cor 50-350 or 50-700 bp size standards loaded in lanes 1, 16, 32, 48 and 64. Known genotypes were loaded in four lanes spread evenly throughout each gel to ensure consistency of allele scores. Microsatellites were referenced to size standards and genotypes were scored using Saga™ GT ver. 3.1 (Lincoln, NE) software. Multi-locus microsatellite genotypes were stored in an Excel™ (Microsoft) spreadsheet for data analysis.

Genetic diversity within populations

Estimates of allele frequency, allele richness (A_r), and observed and expected heterozygosity (H_o , H_e) were computed for each cohort sample. A randomization test was used to test for conformity to Hardy-Weinberg equilibrium for each cohort sample and to test for genotypic disequilibrium among all locus pairs. A G -test of genotypic frequency homogeneity was used to test for genetic differentiation among cohorts within populations pairs. These analyses were performed using the computer program FSTAT version 2.9.3 (Goudet 2001).

Genetic estimates of N_b and N_e

Waples (1990a) showed that the effective population size per generation (N_e) for pacific salmon is equal to the effective number of breeders per year (N_b) multiplied by the mean generation length (g). We used two methods to estimate N_b from genetic data. The first estimate of N_b ($\hat{N}_{b(T)}$) assumes populations are isolated (no gene flow) and was derived using the temporal method modified for Pacific salmon (Waples 1990b). The standardized variance in allele frequency for the j th locus, \hat{F}_j , was computed using the formula

$$\hat{F}_j = \frac{1}{L-1} \sum_{i=1}^L \frac{(X_{i1} - X_{i2})^2}{(X_{i1} + X_{i2})/2},$$

where L is the number of alleles at locus j and X_{i1} and X_{i2} are the frequencies for allele i at the first and second temporal sampling. The weighted mean standardized variance in allele frequency, \hat{F} , was computed using the formula

$$\hat{F} = \frac{\sum[(L_j - 1)\hat{F}_j]}{\sum(L_j - 1)},$$

where L_j is the number of alleles at locus j . Because the adult data was partitioned by cohort, N_b was calculated using the sample plan II formula

$$\hat{N}_b = \frac{b}{\{2[\hat{F} - (1/S)]\}},$$

where b is an empirically derived parameter that reflects the number of generations between the temporal samples (Waples 1990b; Tajima 1992) and S is the harmonic mean of the sample sizes of the two cohorts. This formula was used to compute a single estimate of N_b for each population by computing the average \hat{F} from all pairwise comparisons of temporal samples and then using the weighting scheme proposed by Waples (1990b) to estimate N_b .

The 95% confidence intervals for \hat{F} were computed from a chi-square distribution using the formula

$$\left[\frac{n\hat{F}}{X^2_{.025(n)}}, \frac{n\hat{F}}{X^2_{.975(n)}} \right],$$

where n is equal to $\sum(L_j - 1)$, the number of independent alleles among all loci. These values were used to derive 95% confidence intervals for $\hat{N}_{b(T)}$.

The second estimate of N_b ($\hat{N}_{b(ML)}$) was derived using the computer program MLNE version 1.0 (Wang and Whitlock 2003). This program assumes gene flow among populations and uses maximum likelihood (ML) to simultaneously estimate N_b and the immigration fraction (m) for a single population (the focal population). Immigrants to the focal population are assumed to come from a single source population, however, Wang and Whitlock (2003) showed their ML method will work for other models of gene flow (e.g. island and stepping stone models). They recommend pooling potential source populations into a single sample for analysis. This approach was possible for the Gisasa and Tozitna rivers because the microsatellite loci used in this study were also used to examine eight other Yukon River populations (B. Flannery, USFWS Conservation Genetics Laboratory, unpublished data). Additional data was not available for Kuskokwim River so each population was treated as a source (and temporal samples were

pooled) while the other was focal. The number of generations between each sample (b) was estimated using the program of Tajima (1992). The estimates of b were rounded to the nearest integer as required for input into MLNE version 1.0.

\hat{N}_e was computed from $\hat{N}_{b(T)}$ and $\hat{N}_{b(ML)}$ using the formula

$$\hat{N}_e = g(\hat{N}_b).$$

Demographic estimates of N_b and N_e

Sex-specific census size (annual escapement) data from weirs operated intermittently between 1991 and 2003 was used to determine the impact of fluctuating population size and unequal sex ratio on N_e (Table 2). Data on variation in reproductive success was not available for these populations (or suitable surrogates) so the impact of this factor on N_e was inferred by comparing the N_e estimates based on fluctuating population size and sex ratio with the comprehensive genetic estimate $\hat{N}_{e(T)}$ described above.

Estimates of N_b in year i (\hat{N}_{bi}) were calculated using the formula

$$\hat{N}_{bi} = \frac{4(N_f N_m)}{(N_f + N_m)},$$

described by Wright (1931) where \hat{N}_{bi} is substituted for \hat{N}_e and N_f and N_m are the number of females and males in year i . This formula assumes that the variation in the number of progeny (k) for males and females follows a Poisson distribution (the variance, σ_k^2 , equals the mean, \bar{k}).

Two demographic estimates of N_e were computed. The first estimate of N_e ($\hat{N}_{e(F)}$) accounts for fluctuating population size only and was computed using the formula

$$\hat{N}_{e(F)} = g(\tilde{N}),$$

where \tilde{N} is the harmonic mean of N_i (the total population size year i) values and g is the mean generation length of each population (Waples 2002). Because there is no recruitment data for these populations, we did not use the method of Waples (2002) that accounts for variable

population dynamics when estimating the influence of fluctuating escapement on N_e . The second estimate of N_e ($\hat{N}_{e(FS)}$) adds the sex ratio data and was computed using the formula

$$\hat{N}_{e(FS)} = g(\tilde{N}_b),$$

where \tilde{N}_b is the harmonic mean of \hat{N}_{bi} values.

Estimates of the N_e/N ratio

To further evaluate the relative influence of fluctuating population size, unequal sex-ratio, and variance in reproductive success on N_e , estimates of the ratio of N_e to the arithmetic mean escapement per generation ($g\bar{N}$) were computed for the demographic and genetic estimates of N_e . In order to isolate the influence of sex ratio on N_e , estimates of the ratio $\hat{N}_{e(FS)}/\hat{N}_{e(F)}$ were computed from the two demographic estimators. This ratio is simply the proportional decline in N_e when sex ratio data is combined with data on fluctuating population size, and it is analogous to the index α described by Kalinowski and Waples (2002). To infer the influence of variance in reproductive success on N_e we used the genetic estimator $\hat{N}_{e(T)}$ as a comprehensive N_e estimate and computed the ratio $\hat{N}_{e(T)}/\hat{N}_{e(FS)}$. We assumed that $\hat{N}_{e(T)}$ is smaller than $\hat{N}_{e(FS)}$ because of the influence of variance in reproductive success. Therefore, the ratio $\hat{N}_{e(T)}/\hat{N}_{e(FS)}$ represents the proportional decline in N_e when variance in reproductive success is combined with data on sex ratio and fluctuating population size. With the exception of the Gisasa River, the genetic N_e estimates from the cohort samples do not strictly align temporally with the annual census data (see Waples 2005). Therefore, the genetic $N_e/g\bar{N}$ ratios will be biased because the numerator and denominator reflect slightly different time periods. This bias is not expected to be large, however, because the time periods are close or, in the case of the Kwethluk and Tuluksak, overlap. The N_e/N ratios from this study were compared to N_e/N ratios reported for Pacific salmon (e.g. Shrimpton and Heath 2003; Waples 2004) in order to infer how reliable census size estimates are for evaluating population health of AYK Chinook salmon.

Results

Genetic diversity within populations

The number of alleles per locus ranged from 4 (*Oke4*, *OtsG3*) to 49 (*Oki10*; Appendix 1). The mean allele richness (\bar{A}_r) based on a minimum sample size of 30 individuals was similar among cohorts from the same population (Table 1). The mean value of \bar{A}_r over all cohorts was 10.8. The mean values of expected and observed heterozygosity (\bar{H}_e , \bar{H}_o) over all cohorts were 0.69 and 0.68. The cohort-specific estimates of \bar{A}_r , \bar{H}_e , and \bar{H}_o tended to be lower for the Yukon River Chinook salmon populations (Table 1). A post-hoc analysis using a randomization test in the program FSTAT version 2.9.3 (Goudet 2001) indicated the estimates of \bar{A}_r and \bar{H}_o from the Yukon and Kuskokwim Chinook salmon were significantly different ($P < 0.01$). The randomization tests of conformity to Hardy-Weinberg equilibrium (HWE) and genotypic disequilibrium revealed two cohort samples and 14 locus pairs in which the P -value for the test statistic was below 0.05 (Table 1). These tests were not judged significant when the α -level was adjusted (α/k) for $k = 14$ tests (HWE) and $k = 45$ tests (genotypic disequilibrium) using the sequential Bonferroni method (Rice 1989). The G -test of genotypic frequency homogeneity revealed six pairwise comparisons in which the P -value for the test statistic G was below 0.05. Two cohort samples from the same population (Tuluksak River 1996/1998) were judged significantly different when the α -level was adjusted for (α/k) for $k = 3$ simultaneous tests.

Demographic and genetic N_b

Annual estimates of the effective number of breeders (\hat{N}_{bi}) from the sex ratio data ranged from 545 (Tuluksak River, 1992) to 8,982 (Kwethluk River, 2003, Table 2). The ratio $\hat{N}_{bi(S)}/N_i$ ranged from 0.45 (Tozitna River 2002) to 1.00 (Gisasa River 2001) and averaged 0.71 over all years and populations.

The two genetic estimators provided different values of \hat{N}_b (Table 3). The estimates that assume isolation ($\hat{N}_{b(T)}$) ranged from 460 (Tozitna River) to 1,619 (Kwethluk River). The upper bound of the 95% confidence interval for each $\hat{N}_{b(T)}$ was negative, indicating the sampling variance is relatively high for this time period. The estimates that assume migration ($\hat{N}_{b(ML)}$)

ranged from 92 (Tozitna River) to 224 (Kwethluk River, Table 3). The maximum likelihood estimates of the immigration fraction ($\hat{m}_{(ML)}$) ranged from 0.07 to 0.09 for the Tozitna and Gisasa rivers and from 0.34 to 0.43 for the Kwethluk and Tuluksak rivers (Table 3).

Demographic and genetic N_e

Two demographic estimates of N_e were computed for each population for time periods ranging from 2 years (Tozitna River) to 12 years (Tuluksak River, Table 4). Two time periods were examined for the Gisasa River population so that a direct comparison could be made with the genetic estimates of N_e from the 1995-97 cohort. The largest values of \hat{N}_e reflected only fluctuating escapement ($\hat{N}_{e(F)}$), ranging from 6,342 (Tuluksak River) to 32,661 (Kwethluk River). These values declined when sex ratio data was added. The values of $\hat{N}_{e(FS)}$ ranged from 4,247 (Tuluksak River) to 22,321 (Kwethluk River). The genetic estimates of N_e that assume isolation ($\hat{N}_{e(T)}$) exhibited greater variation among populations than did the estimates that assume migration ($\hat{N}_{e(ML)}$, Table 4). $\hat{N}_{e(T)}$ ranged from 2,307 (Tozitna River) to 7,674 (Kwethluk River) and $\hat{N}_{e(ML)}$ ranged from 448 (Kwethluk River) to 576 (Tozitna River).

N_e/N ratio

The demographic estimates of the ratio $N_e/g\bar{N}$ that consider only fluctuating population size ($\hat{N}_{e(F)}/g\bar{N}$) ranged from 0.77 (Kwethluk River) to > 0.99 (Tozitna River) and averaged 0.88 (Table 5). When the sex ratio data was added ($\hat{N}_{e(FS)}/g\bar{N}$) the values declined, ranging from 0.50 (Tozitna River) to 0.70 (Gisasa River) and averaging 0.58. . The mean genetic estimates ranged from 0.28 ($\hat{N}_{e(T)}/g\bar{N}$), assuming isolation, to 0.04 ($\hat{N}_{e(ML)}/g\bar{N}$), assuming migration. Estimates of the ratio $\hat{N}_{e(FS)}/\hat{N}_{e(F)}$ ranged from 0.50 (Tozitna River) to 0.75 (Gisasa River), and estimates of the ratio $\hat{N}_{e(T)}/\hat{N}_{e(FS)}$ ranged from 0.33 (Gisasa River) to 0.83 (Tuluksak River).

Discussion

The objectives of this study were to estimate and evaluate N_e and the relative influence of demographic variables on N_e of Chinook salmon in the Kwethluk, Tuluksak, Gisasa and Tozitna rivers. The four major findings are: 1) the genetic estimates of N_e that assume isolation ($\hat{N}_{e(T)}$) range from 2,307 to 7,674; 2) the genetic estimates of N_e that assume migration ($\hat{N}_{e(ML)}$) range from 448 to 576, and the companion estimates of the immigration fraction ($\hat{m}_{(ML)}$) range from 0.07 to 0.09 in the Yukon River tributaries and 0.34 to 0.43 in the Kuskokwim River tributaries; 3) the average $\hat{N}_e/g\bar{N}$ ratios are 0.04 and 0.28 for the genetic estimators $\hat{N}_{e(ML)}/g\bar{N}$ and $\hat{N}_{e(T)}/g\bar{N}$; and 4) the genetic and demographic estimators of N_e/N suggest N_e is influenced most by unequal sex ratio and variance in reproductive success and, and least by fluctuating population size for the time periods examined in each population.

Genetic estimates of N_e without immigration

Estimates of N_e can be used as a measure of the genetic health of a population. While there are no strict criteria for evaluating N_e , values of 50 and 500 are considered general thresholds indicating significant threat of short- and long-term loss of genetic diversity in isolated populations (Waples 1990a; Mace and Lande 1991; Allendorf et al. 1997). The genetic estimates that assume isolation ($\hat{N}_{e(T)}$) are all well above the threshold of concern ($N_e \approx 500$) for long term loss of genetic diversity in isolated populations.

For perspective, these estimates are substantially larger than those of Shrimpton and Heath (2003) for five upper Fraser River Chinook salmon populations with mean annual adult returns similar to the populations in this study. Using the temporal method, Shrimpton and Heath (2003) report estimates of N_e that are at or below 500. Three factors, either alone or collectively, could explain the differences in $\hat{N}_{e(T)}$ between the Western Alaska and upper Fraser River Chinook salmon populations. First, Shrimpton and Heath (2003) show a positive correlation between available spawning habitat and N_e , and they suggest access to appropriate spawning sites is limiting N_e even as the census size increases. In contrast, there is no evidence that spawning habitat is similarly limiting abundance of the Western Alaska Chinook salmon populations in this study.

Shrimpton and Heath (2003) also examine a longer time period (20 years) and suggest that evidence of population bottlenecks in the 1980s may explain the low N_e estimates of some upper Fraser River Chinook salmon. Three of the five upper Fraser River populations recorded annual adult returns below 160 between 1980 and 1998. Escapement data is limited for Western Alaska Chinook salmon, however, there is no indication that these populations have recently undergone similar severe declines. With the exception of the Tuluksak River in 1991, recorded annual adult returns have all been greater than 1,000 (Table 2).

Finally, it is possible that migration may be influencing the allele frequencies in the Western Alaska populations to a much greater degree than in the upper Fraser River populations. The temporal method assumes populations are isolated, however, regular migration will cause allele frequencies to change more slowly and bias the N_e estimate upwards (Waples 1990b). Although the temporal method is believed to be robust to immigration rates in most natural salmon populations, it may be sufficiently large in Western Alaska Chinook salmon to bias N_e . One indication that this is the case is the level of genetic differentiation among populations as indicated by the index F_{st} . Because F_{st} is inversely proportional to the effective number of migrants ($N_e m$), F_{st} estimates can be used to infer the relative magnitude of migration within different groups of populations if it is assumed the population size is similar among the groups. The Yukon and Kuskokwim River populations exhibit relatively low values of F_{st} (0.011 and 0.002 respectively) compared to upper Fraser River Chinook salmon (0.017, Beacham et al. 2003), suggesting migration rate could be higher among the Alaska populations. Interestingly, the N_e estimates from this study that assume immigration (see below) are similar to the N_e estimates of Shrimpton and Heath (2003) that assume a closed population.

Genetic estimates of N_e with immigration

The genetic $\hat{N}_{e(ML)}$ is based on a migration model that assumes the population of interest (focal population) receives immigrants each generation from a single, infinitely large, source population. This assumption is unrealistic, but Wang and Whitlock (2003) show by simulation that the model is robust and will provide satisfactory estimates of N_e and m for other patterns of migration (e.g. island and stepping stone models). In this study, the maximum likelihood method of Wang and Whitlock (2003) provided genetic estimates of N_e near the threshold of concern ($N_e \approx 500$) for long term loss of genetic diversity in isolated populations. These $\hat{N}_{e(ML)}$ values are

between 4 and 17 times smaller than the corresponding $\hat{N}_{e(T)}$ values, however, the companion estimates of the immigration fraction ($\hat{m}_{(ML)}$) indicate intra-population genetic diversity may be influenced substantially by gene flow.

There is little data on rates of gene flow in Pacific salmon with which to evaluate these results but the limited estimates of rates of straying (not gene flow) suggest the degree of movement among populations can vary widely within species (reviewed by McElhany et al. 2000). In this context we consider the possible implications of the $\hat{m}_{(ML)}$ values with respect to the genetic diversity and health of Yukon and Kuskokwim River Chinook salmon. First, the results imply that migration is a key determinant of genetic diversity in these populations and that their long term genetic health may depend upon persistent gene flow. Closed populations with an N_e near 500 merit conservation concern, however, it is likely that the populations in this study are interconnected as, for example, a metapopulation like that proposed by Cooper and Mangel (1999) for Pacific salmon (see also Wainwright and Waples 1998). In this source-sink model, connectivity prevents extinction or loss of genetic diversity of sink populations and the relationship (source to sink) is dynamic and changes as the environment evolves. Maintaining connectivity and habitat health in this type of system is critical to long term population health.

The results also imply that the rate of gene flow can vary substantially among populations. In the present study, the middle Yukon and lower Kuskokwim River populations appear to exhibit very different immigration rates (Yukon River $\hat{m}_{(ML)} < 10\%$; Kuskokwim River $\hat{m}_{(ML)} > 30\%$) despite having similar $\hat{N}_{e(ML)}$ values. This difference may reflect important regional variation that influences population evolution and vulnerability to loss of diversity. McElhany et al. (2000) suggested a population could be considered demographically independent if the immigration rate is less than 10% as is the case for the Gisasa and Tozitna River Chinook salmon. This threshold is based on a simulation study (Hastings 1993) and has not been tested empirically in Pacific Salmon, however, the $\hat{m}_{(ML)}$ values suggest the two Yukon River populations are less influenced by gene flow and may be more vulnerable to loss of genetic diversity and extinction than the lower Kuskokwim River populations. Selection may favor relatively high migration rates in the dynamic, low gradient, streams of the lower Yukon and Kuskokwim River Delta region (Nowacki et al. 2002) whereas local adaptation may promote stronger homing by Chinook

salmon in the middle Yukon River.

Finally, it should be noted that the Wang and Whitlock (2003) method has not been fully evaluated in Pacific salmon and further analysis is needed to verify the findings and conclusions above. We feel, however, the values of $\hat{m}_{(ML)}$ for this study are reasonable given the level of genetic population structure in Chinook salmon from the Yukon and Kuskokwim River (e.g. Templin et al. 2004; B. Flannery unpublished data). To illustrate this we show that the estimates of F_{st} from the formula $F_{st} = 1/(4N_e m + 1)$ where $\hat{N}_{e(ML)}$ and $\hat{m}_{(ML)}$ are used to derive $N_e m$ are similar to the observed values of F_{st} for the Yukon and Kuskokwim River population pairs. The estimates of F_{st} from the $\hat{N}_{e(ML)}$ and $\hat{m}_{(ML)}$ values (with 95% confidence intervals) for the Gisasa and Tozitna rivers are 0.006 (0.002 – 0.022) and 0.007 (0.002 – 0.025). The observed F_{st} is 0.011. The estimates of F_{st} from the $\hat{N}_{e(ML)}$ and $\hat{m}_{(ML)}$ values for the Kwethluk and Tuluksak rivers are 0.002 (0.001 – 0.003) and 0.001 (0.001 – 0.002). The observed F_{st} is 0.002.

Genetic estimates of the N_e/N ratio

Comprehensive estimates of N_e are often difficult to obtain because complete demographic or genetic data is not available. As an alternative, average estimates of the ratio N_e/N from “similar populations” can be combined with estimates of census size per generation (for salmon, the arithmetic mean adult count per generation, $g\bar{N}$) to derive an estimate of N_e for the population of interest (e.g. Frankham 1995). The two genetic estimators in this study ($\hat{N}_{e(T)}/g\bar{N}$ and $\hat{N}_{e(ML)}/g\bar{N}$) reflect the ratio of comprehensive N_e to $g\bar{N}$ for four Yukon and Kuskokwim River Chinook salmon populations. The mean estimates range from 0.04 ($\hat{N}_{e(ML)}/g\bar{N}$) to 0.28 ($\hat{N}_{e(T)}/g\bar{N}$) and clearly show that census size is not an accurate indicator of genetic health in these populations. The disparity in mean estimates reflect the different underlying assumptions regarding gene flow used in estimating N_e .

The population-specific estimates vary as well. For example, the estimates $\hat{N}_{e(T)}/g\bar{N}$ that assume isolation vary from 0.18 to 0.44 (CV = 0.43). Three of the four values are within the range (0.05 – 0.30) describe by Waples (2004) for other Chinook salmon populations. The estimates ($\hat{N}_{e(ML)}/g\bar{N}$) that assume migration are much smaller, but the values (0.01 – 0.07)

exhibit even greater relative variance (CV = 0.64). This level of inter-population variation is not surprising given the results of other studies of Pacific salmon. Heath et al. (2002) and Shrimpton and Heath (2003) found that N_e/N estimates (assuming isolation) for upper Fraser River Chinook salmon and northern British Columbia steelhead (*Oncorhynchus mykiss*) ranged from 0.02 to 0.22 and 0.06 to 0.29, respectively. These findings and those of this study have two implications. First, surrogate estimates of N_e/N can have relatively low precision even when derived from populations in the same region as the population for which N_e is to be estimated. Therefore, if possible, estimates of N_e derived from surrogate N_e/N estimates should be accompanied with indicators of variance (e.g. \pm SD). Second, N_e/N ratios can vary substantially, even on a relatively fine geographic scale. In this study, the $\hat{N}_{e(T)}/g\bar{N}$ values for Chinook salmon from the Kwethluk and Tuluksak rivers are 0.18 and 0.44, respectively, despite the fact that the two river mouths are only 60 km apart on the lower Kuskokwim River. These results suggest that demographic factors may be impacting the two populations differently despite their relative close proximity (see below).

Sufficient demographic and genetic data is not available to estimate N_e for most Chinook salmon populations in the Yukon and Kuskokwim rivers. For many of these populations, however, there are some estimates of total return that may be combined with the mean N_e/N values from this study to estimate N_e provided an indication of the variance (\pm SD) accompanies each estimate. Because the migration rates may vary substantially, it seems reasonable to use the mean $\hat{N}_{e(T)}/g\bar{N}$ (0.28 ± 0.12) and be aware that estimates of N_e under the assumption of isolation may be much larger than estimates assuming migration. Populations with estimates of N_e near or below 500 using this approach (i.e. assuming isolation) should be examined closely and, if possible, genetic data should be used to estimate N_e and m . These estimates will determine the degree to which migration is influencing genetic diversity in these small populations.

Influence of demographic variables on N_e

Estimates of the N_e/N ratio are also useful for inferring the relative influence of demographic variables on N_e (Kalinowski and Waples 2002; Ardren and Kapuscinski 2003). In this context, the genetic estimator $\hat{N}_{e(T)}/g\bar{N}$ provides a comprehensive “yardstick” with which to evaluate the demographic estimators $\hat{N}_{e(F)}/g\bar{N}$ and $\hat{N}_{e(FS)}/g\bar{N}$ because they all assume a closed population.

As stated in the methods, the genetic and demographic estimators of N_e reflect slightly different time periods for three of the four populations, however, the time period differences are small enough to allow general comparison.

The comparison of $\hat{N}_{e(T)}/g\bar{N}$ (mean 0.28, range 0.18 – 0.44) to $\hat{N}_{e(F)}/g\bar{N}$ (mean 0.88, range 0.77 – 1.00) indicates that fluctuating escapement has relatively little influence on N_e . This result not surprising given the short time period examined. Over longer periods (say > 20 years), annual adult returns of salmon fluctuate more and these fluctuations will likely include years of low abundance that have a greater effect on N_e . For example, the mean estimate of the ratio of harmonic mean return to arithmetic mean return (analogous to our $\hat{N}_{e(F)}/g\bar{N}$) for upper Fraser River Chinook salmon was 0.63 (range 0.31 – 0.86, Shrimpton and Heath 2003). It should also be noted that the estimator $\hat{N}_{e(F)}/g\bar{N}$ does not include recruitment data because it is not available for these populations. Waples (2002) described an N_e/N estimator that uses recruitment data (not variance in reproductive success) when determining the impact of fluctuating escapement. This estimate was often smaller than the N_e/N estimate based on the harmonic mean.

The estimates of $\hat{N}_{e(FS)}/\hat{N}_{e(F)}$ represent the proportional decline in N_e when sex ratio data is combined with data on fluctuating population size. These values (0.50 – 0.75) are smaller than the corresponding estimates of $\hat{N}_{e(F)}/g\bar{N}$, indicating that unequal sex ratio is impacting N_e to a much greater degree than fluctuating population size. However, the estimates of $\hat{N}_{e(T)}/g\bar{N}$ suggest the relative influence of sex ratio on N_e varies substantially among populations. For example, the Tuluksak and Kwethluk River populations exhibit similar values of $\hat{N}_{e(FS)}/\hat{N}_{e(F)}$ but different estimates of $\hat{N}_{e(T)}/g\bar{N}$ and $\hat{N}_{e(T)}/\hat{N}_{e(FS)}$ (the proportional decline in N_e when variance in reproductive success is combined with data on sex ratio and fluctuating population size). The relatively small $\hat{N}_{e(T)}/\hat{N}_{e(FS)}$ estimate for the Kwethluk River Chinook salmon (0.34) suggests variance in reproductive success has a large impact on N_e in this population. In contrast, the estimate of $\hat{N}_{e(T)}/\hat{N}_{e(FS)}$ for the Tuluksak River Chinook salmon (0.83) is relatively large, suggesting N_e in this population is influenced most by unequal sex ratio. Because the two populations exhibit similar sex ratios (22% female) for the time periods studied, these results

suggest the variance in reproductive success is correlated with the annual adult return. The arithmetic mean adult count per year (\bar{N}) in the Kwethluk River is 5.8 times larger ($\bar{N} = 9,001$) than in the Tuluksak River ($\bar{N} = 1,554$). There is some evidence that the N_e/N ratio is inversely related to census size within a population (Pray et al. 1996; Ardren and Kapuscinski 2003). This relationship in salmon may be the result of compensatory mortality. Ardren and Kapuscinski (2003) describe some biological mechanisms, including territoriality in females and redd superimposition, that likely link an increase in variance in reproductive success with an increase in N (and a decline in N_e/N). The estimates of $\hat{N}_{e(FS)}/\hat{N}_{e(F)}$ and $\hat{N}_{e(T)}/\hat{N}_{e(FS)}$ suggest both populations are impacted similarly by unequal sex ratio but that one or more of the above mechanisms is influencing Kwethluk River Chinook salmon to a greater degree than Tuluksak River Chinook salmon because more adults are returning to the Kwethluk River.

The estimate of $\hat{N}_{e(T)}/\hat{N}_{e(FS)}$ is smaller for the Gisasa River Chinook salmon (0.33) than for the Tozitna River Chinook salmon (0.54). In this case, however, the two populations exhibit similar estimates of $\hat{N}_{e(T)}/g\bar{N}$, suggesting sex ratio and variance in reproductive success impact N_e differently in each population but their combined impact is about the same. The average sex ratio for Chinook salmon in the Gisasa and Tozitna rivers is 30% and 15%, respectively. Sex ratio clearly has a larger impact on N_e of Chinook salmon in the Tozitna River as indicated by the fact that $\hat{N}_{e(FS)}/\hat{N}_{e(F)}$ (0.50) is smaller than $\hat{N}_{e(T)}/\hat{N}_{e(FS)}$ (0.54). In contrast, variance in reproductive success appears to have a greater impact on N_e of Gisasa River Chinook salmon. The ratio $\hat{N}_{e(T)}/\hat{N}_{e(FS)}$ (0.33) is much smaller than $\hat{N}_{e(FS)}/\hat{N}_{e(F)}$ (0.71). As hypothesized above, the influence of variance in reproductive success on N_e may be related to the number of adults returning to each river. The Gisasa River has the second largest adult return of Chinook salmon (for the years sampled in this study). It's run size, however, is not that much larger than the Tozitna River and these results may simply indicate that, at the current level of adult returns in both rivers, the Chinook salmon in the Gisasa River exhibit greater variance in reproductive success.

The results support a conclusion that the N_e of these Chinook salmon populations is influenced most by unequal sex ratio and variance in reproductive success and least by fluctuating population size for the time period examined in this study. The relative influence of unequal sex

ratio and variance in reproductive success on N_e differs among populations. Variance in reproductive success has the largest impact in the populations with the largest annual adult returns (Kwethluk and Gisasa rivers). Sex ratio appears to have the largest impact in the population with the smallest annual return (Tuluksak River) and the population with the most extreme sex ratio bias (Tozitna River). These results suggest that sex ratio bias, more than variance in reproductive success, will likely effect N_e at the threshold ($N_e \leq 500$) for long term loss of genetic diversity.

Summary and recommendations

In summary for the Chinook salmon populations examined in this study:

- The estimates of effective population size that assume isolation, $\hat{N}_{e(T)}$, are well above the threshold of concern ($N_e \approx 500$) for long term loss of genetic diversity.
- The estimates of effective population size that assume migration, $\hat{N}_{e(ML)}$, are near 500, but the companion estimates of the immigration fraction ($\hat{m}_{(ML)}$) suggest gene flow is an important factor in maintaining genetic diversity.
- The estimates of the immigration fraction $\hat{m}_{(ML)}$ appear to be greater among the lower Kuskokwim River Chinook salmon than among the middle Yukon River Chinook salmon.
- The mean ratio of effective population size to the arithmetic mean adult count per generation, $\hat{N}_{e(T)}/g\bar{N}$, is 0.28, assuming isolation. The mean ratio $\hat{N}_{e(ML)}/g\bar{N}$ is 0.04, assuming migration. The estimates of both ratios vary widely among populations (CV = 0.43 and 0.64, respectively).
- N_e appears to be influenced most by sex ratio and variance in reproductive success for the time period of this study. The degree of influence of each factor varies among the populations. Variance in reproductive success appears to have the greatest impact in the populations with the largest census size.

These points support the following recommendations:

- Conservation and management actions should maintain connectivity among populations because gene flow appears to be an important factor in maintaining genetic diversity. For

example, land use planners should avoid habitat impacts that alter salmon migration patterns and fishery managers must be aware that harvest strategies may alter gene flow patterns as well as the escapement number.

- Sex ratio should continue to be monitored because its impact on N_e will likely increase if annual adult returns decline. Long term monitoring of these rivers is recommended to provide accurate escapement numbers and sex ratio data.
- The mean ratio $\hat{N}_{e(T)}/g\bar{N}$ ratio (0.28) can be used as a surrogate with escapement data to estimate N_e of other Yukon and Kuskokwim River Chinook salmon populations provided an indication of the variance (\pm SD) accompanies each estimate. Estimates of N_e from $\hat{N}_{e(T)}/g\bar{N}$ assume isolation and may be much larger than estimates assuming migration. Therefore, populations with estimates of N_e near or below 500 using this approach (i.e. assuming isolation) should be examined closely and, if possible, genetic data should be used to estimate N_e and m .
- A larger study is warranted to evaluate gene flow patterns and rates among populations from different regions in each watershed. Such a study could identify genetically vulnerable populations as well as test hypotheses about the type of population structure and important factors (e.g. landscape features and habitat) that influence genetic diversity and vulnerability. Surrogate measures of available habitat for spawning and rearing could be used to test hypotheses about the relationship between total adult return and variance in reproductive success.

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Deliverables

The following deliverables disseminate the findings from this study: 1) two progress reports and a final report available through the AYKSSI program or from the authors; 2) an oral report titled “The role of gender in biocomplexity: male-biased sex ratios and effective population size in Chinook salmon” presented at the 2005 annual meeting of the American Fisheries Society in Anchorage, Alaska; 3) a manuscript in preparation for submission to a peer-reviewed journal; 4) genotype and allele frequency data in a Microsoft Excel spreadsheet available from the authors and on the Conservation Genetics Website (<http://alaska.fws.gov/fisheries/genetics/data.htm>); 5) a manuscript in preparation for submission to a peer-reviewed journal.

Project Data

The primary data for this study is the individual genotype data (genotype for each locus and individual) for each cohort and the demographic data (escapement data by gender for each river). This data is available from the authors in an Excel spreadsheet (Microsoft Office version 11). Allele frequency data for each cohort is available in Appendix 1 of this report and on the Conservation Genetics Website (<http://alaska.fws.gov/fisheries/genetics/data.htm>). The primary author can be contacted at:

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Press Release

Maintaining genetic diversity is necessary for maintaining healthy, viable populations. This tenet of conservation is most relevant for populations that are small or are experiencing significant declines in abundance. Small populations are of particular concern because loss of genetic diversity is inversely proportional to population abundance. In this context, abundance refers to the effective size of the population (N_e), not the census size (N), and theory suggests genetic diversity is lost at a rate equal to $1/(2N_e)$ per generation. Thus, the N_e is an important indicator of the genetic health and viability of a population. Conservation guidelines have been established from theoretical studies that suggest isolated populations having an N_e below 500 (50) are at risk of significant long-term (short-term) loss of genetic diversity. These threshold values of 500 and 50 provide a yardstick with which to evaluate N_e estimates.

In this study, genetic analysis was used to estimate contemporary N_e for Chinook salmon from the Gisasa and Tozitna rivers, tributaries of Yukon River, and the Kwethluk and Tuluksak rivers, tributaries of the Kuskokwim River. Two N_e estimates were derived for each population: one assuming isolation and the other assuming the populations are connected by migration. The estimates of N_e that assume isolation range from 2,307 to 7,674 and the estimates that assume immigration range from 448 to 576. Estimates of the rate of immigration vary by region and are four to five times greater in the lower Kuskokwim River than in the middle Yukon River. Combining the genetic data and demographic data suggests N_e is influenced most by unequal sex ratio and variation in individual reproductive success. These results indicate the observed sex ratio bias in these populations could adversely impact the long term genetic health if annual run sizes and migration rates decline. Further, the results underscore the importance of maintaining population connectivity and suggest that the vulnerability to loss of genetic diversity varies regionally and is negatively correlated with the rate of immigration from neighboring populations.

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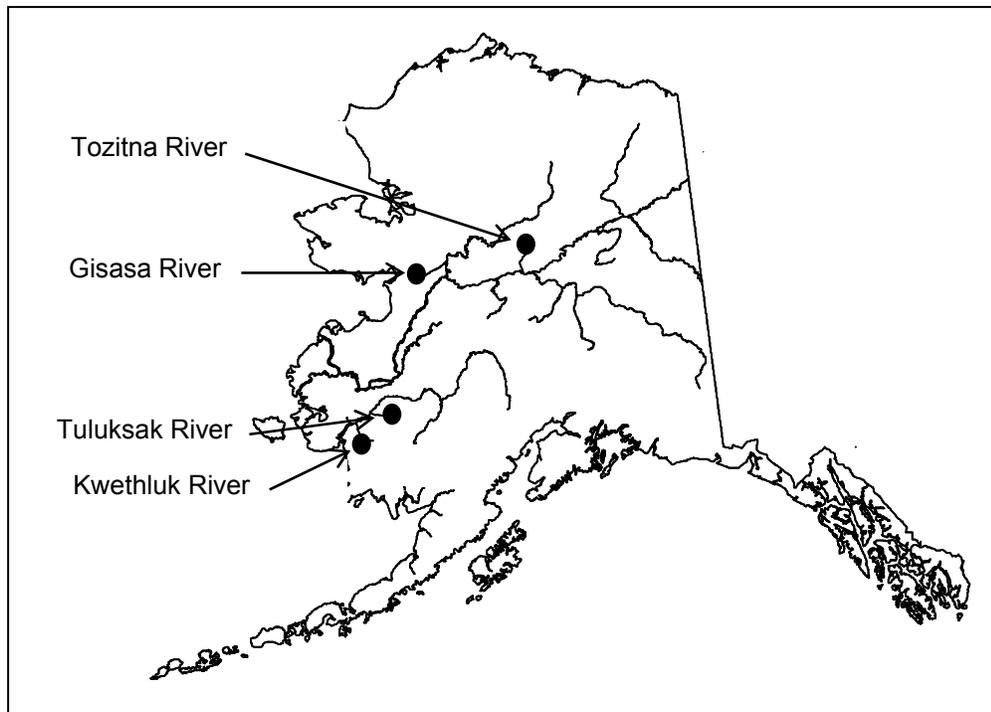


Figure 1. Map of Alaska showing the Kwethluk, Tuluksak, Gisasa, and Tozitna rivers.

Table 1. Summary of sample data for Chinook salmon from the Yukon and Kuskokwim rivers^a.

Drainage/Sample	LS	Year	Cohort	n	\bar{A}_r	\bar{H}_e	\bar{H}_o	f
Yukon R.								
Gisasa R.	A	2001	1995	124	10.3	0.68	0.66	0.023
			1996	72	10.6	0.69	0.68	0.013
			1997	56	10.9	0.68	0.67	0.019
Tozitna R.	A	2002/03	1997	123	9.9	0.69	0.66	0.028*
			1998	190	9.9	0.68	0.67	0.006
			1999	54	9.6	0.68	0.64	0.041*
Kuskokwim R.								
Tuluksak R.	A	2002/03	1996	55	11.1	0.68	0.68	-0.005
			1997	83	11.2	0.69	0.70	-0.015
			1998	81	11.0	0.70	0.69	0.005
Kwethluk R.	J	1990	1989	96	11.1	0.69	0.67	0.023
			1996	69	11.4	0.70	0.70	0.000
	A	2002	1997	116	11.3	0.70	0.70	-0.003
			1998	155	11.2	0.68	0.68	-0.003
			2002	80	11.8	0.70	0.70	-0.006

^a LS – life history stage, A – adult, J – juvenile; Year – sample year; Cohort – individuals of same age; n – sample size; \bar{A}_r – allele richness per locus; \bar{H}_e – expected heterozygosity per locus; \bar{H}_o – observed heterozygosity per locus; f – index of heterozygote deficit. An asterisk (*) indicates $P < 0.05$ that the sample conforms to Hardy-Weinberg expectation.

Table 2. Estimates of the effective number of breeders (\hat{N}_{bi}) from sex ratio data^a.

Sample/year	N_i	N_m	N_f	\hat{N}_{bi}	R
Gisasa R.					
1995	4023	2174	1849	3997	0.99
1996	1952	1571	381	1226	0.63
1997	3764	2888	876	2689	0.71
1998	2356	1954	402	1334	0.57
1999	2631	1876	755	2153	0.82
2000	2089	1455	634	1766	0.85
2001	3052	1539	1513	3052	1.00
2002	1931	1521	410	1292	0.67
2003	1852	1158	694	1736	0.94
\tilde{X}	2436			1838	
Tozitna R.					
2002	1596	1392	204	712	0.45
2003	1819	1492	327	1073	0.59
\tilde{X}	1700			856	
Tuluksak R.					
1991	697	496	201	572	0.82
1992	1083	923	160	545	0.50
1993	2218	1911	307	1058	0.48
1994	2917	2226	691	2109	0.72
2002	1346	1028	318	971	0.72
2003	1064	776	288	840	0.79
\tilde{X}	1241			831	
Kwethluk R.					
1992	9675	7275	2400	7219	0.75
2000	3461	2695	766	2386	0.69
2002	8395	6644	1751	5543	0.66
2003	14474	11695	2779	8982	0.62
\tilde{X}	6891			4709	

^a N_i – total number of adults; N_m, N_f – total number of males and females; $R = \hat{N}_{bi}/N_i$ ratio; \tilde{X} – harmonic mean.

Table 3. Estimates of the effective number of breeders per year (\hat{N}_b) from genetic data^{a,b}.

Sample/years	$\hat{N}_{b(T)}$ (95%CI)	$\hat{N}_{b(ML)}$ (95%CI)	$\hat{m}_{(ML)}$ (95%CI)
Gisasa R.			
95-97	695 (203-∞)	94 (65-154)	0.09 (0.03-0.16)
Tozitna R.			
97-99	460 (187-∞)	115 (85-166)	0.07 (0.02-0.12)
Tuluksak R.			
96-98	691 (194-∞)	92 (68-133)	0.43 (0.30-0.57)
Kwethluk R.			
89,96-98,02	1619 (322-∞)	95 (78-137)	0.34 (0.24-0.45)

^a The temporal method of Waples (1990, $\hat{N}_{b(T)}$) assumes populations are isolated.

^b The maximum likelihood method of Wang and Whitlock (2003) assumes gene flow and computes estimates of $\hat{N}_{b(ML)}$ and the immigration fraction ($\hat{m}_{(ML)}$) simultaneously.

Table 4. Average adult count^a and estimates of the effective population size per generation (\hat{N}_e) from demographic^b and genetic data^c.

Sample/years	\bar{N}	\tilde{N}	$g\bar{N}^d$	Demographic		Genetic	
				$\hat{N}_{e(F)}$	$\hat{N}_{e(FS)}$	$\hat{N}_{e(T)}$ (95%CI)	$\hat{N}_{e(ML)}$ (95%CI)
Gisasa R.							
95-97	3246	2922	16978	15284	10911	3634 (1062-∞)	491 (340-804)
95-03	2628	2436	13743	12738	9614		
Tozitna R.							
97-99						2307 (940-∞)	576 (427-834)
02-03	1708	1700	8572	8535	4296		
Tuluksak R.							
96-98						3532 (991-∞)	470 (348-677)
91-94,02-03	1554	1241	7942	6342	4247		
Kwethluk R.							
89,96-98,02						7674 (1527-∞)	448 (368-649)
92,00,02-03	9001	6891	42666	32661	22321		

^a \bar{N} – arithmetic mean adult count per year; \tilde{N} – harmonic mean adult count per year; $g\bar{N}$ – arithmetic mean adult count per generation.

^b Demographic estimates of \hat{N}_e reflect fluctuations in population size ($\hat{N}_{e(F)}$); and fluctuations in population size plus unequal sex ratio ($\hat{N}_{e(FS)}$).

^c Genetic estimates of \hat{N}_e were computed using the temporal method of Waples (1990, $\hat{N}_{e(T)}$) and the maximum likelihood method of Wang and Whitlock (2003, $\hat{N}_{e(ML)}$).

^d Estimates of mean generation time (g) are 5.23 (Gisasa); 5.02 (Tozitna); 5.11 (Tuluksak); 4.74 (Kwethluk).

Table 5. Estimates of the ratio of effective population size to the arithmetic mean adult count per generation^a. The ratios $\hat{N}_{e(FS)}/\hat{N}_{e(F)}$ and $\hat{N}_{e(T)}/\hat{N}_{e(FS)}$ are estimates of the relative influence of sex ratio and variance in reproductive success, respectively, on N_e .

Sample/years	Demographic ^b			Genetic ^{c,d}		
	$\hat{N}_{e(F)}/g\bar{N}$	$\hat{N}_{e(FS)}/g\bar{N}$	$\hat{N}_{e(FS)}/\hat{N}_{e(F)}$	$\hat{N}_{e(T)}/g\bar{N}$	$\hat{N}_{e(T)}/\hat{N}_{e(FS)}$	$\hat{N}_{e(ML)}/g\bar{N}$
Gisasa R.						
95-97	0.90	0.64	0.71	0.21	0.33	0.03
95-03	0.93	0.70	0.75			
Tozitna R.						
97-99				0.27	0.54	0.07
02-03	>0.99	0.50	0.50			
Tuluksak R.						
96-98				0.44	0.83	0.06
91-94,02-03	0.80	0.53	0.67			
Kwethluk R.						
89,96-98,02				0.18	0.34	0.01
92,00,02-03	0.77	0.52 (0.68)	0.68			

^a $g\bar{N}$ – arithmetic mean adult count per generation.

^b Demographic estimates of \hat{N}_e reflect fluctuations in population size ($\hat{N}_{e(F)}$), and fluctuations in population size plus unequal sex ratio ($\hat{N}_{e(FS)}$).

^c Genetic estimates of \hat{N}_e were computed using the temporal method of Waples (1990, $\hat{N}_{e(T)}$) and the maximum likelihood method of Wang and Whitlock (2003, $\hat{N}_{e(ML)}$).

^d Estimates of $g\bar{N}$ did not correspond to genetic sample years for the Tozitna, Tuluksak and Kwethluk rivers.

Appendix 1. Table of allele frequencies at each locus for each cohort from the four populations. A dash indicates the allele was not present.

Locus Allele	<u>Gisasa R.</u>			<u>Tozitna R.</u>			<u>Tuluksak R.</u>				<u>Kwethluk R.</u>			
	1995	1996	1997	1997	1998	1999	1996	1997	1998	1989	1996	1997	1998	2002
Oke2														
165	---	---	---	---	---	---	---	---	---	---	---	---	---	0.006
169	---	---	---	---	---	---	---	---	---	---	---	---	---	0.013
171	---	---	---	---	---	---	---	---	---	---	---	---	---	0.006
177	---	---	---	---	---	---	---	---	---	---	---	---	0.006	---
181	---	---	---	---	---	---	---	---	---	0.011	---	---	---	---
183	0.040	0.035	0.045	0.033	0.039	0.046	0.082	0.060	0.037	0.064	0.051	0.056	0.068	0.071
185	0.129	0.092	0.055	0.175	0.168	0.130	0.082	0.120	0.099	0.090	0.103	0.112	0.090	0.117
187	0.069	0.085	0.036	0.037	0.045	0.046	0.045	0.048	0.012	0.048	0.037	0.052	0.032	0.032
189	0.218	0.176	0.273	0.110	0.118	0.111	0.109	0.139	0.136	0.181	0.279	0.228	0.203	0.188
191	0.089	0.085	0.091	0.146	0.113	0.111	0.091	0.090	0.111	0.122	0.074	0.099	0.116	0.123
193	0.198	0.303	0.200	0.134	0.161	0.111	0.209	0.235	0.235	0.202	0.206	0.172	0.223	0.175
195	0.105	0.063	0.091	0.106	0.108	0.148	0.082	0.078	0.074	0.043	0.066	0.069	0.071	0.091
197	0.020	0.007	0.009	0.004	0.008	0.009	0.055	0.030	0.043	0.021	0.007	0.039	0.032	0.019
199	0.004	---	0.009	0.016	0.018	---	0.018	0.042	0.043	0.027	0.015	0.013	0.019	0.006
201	0.024	0.056	0.055	0.012	0.021	0.009	0.027	0.024	0.012	0.059	0.022	0.039	0.013	0.026
203	0.081	0.077	0.082	0.224	0.200	0.269	0.145	0.084	0.117	0.112	0.096	0.103	0.116	0.097
205	---	---	---	---	---	---	---	---	---	---	---	0.004	---	0.006
207	---	---	---	---	---	---	---	---	0.012	---	0.007	0.004	---	---
209	0.008	0.007	0.036	---	---	0.009	---	0.006	0.019	---	0.007	0.009	0.003	---
213	0.016	0.014	0.018	0.004	---	---	0.036	0.036	0.049	0.021	0.029	---	0.006	0.019
215	---	---	---	---	---	---	0.018	0.006	---	---	---	---	---	---
Ots3.1														
97	0.008	0.014	0.019	---	---	---	---	---	---	---	---	---	---	---
109	---	---	---	---	---	---	---	---	0.006	---	---	---	---	---
113	0.167	0.190	0.222	0.143	0.122	0.148	0.136	0.096	0.154	0.146	0.138	0.171	0.116	0.128
115	0.012	0.007	0.009	---	---	---	---	---	---	---	---	---	---	0.006
117	0.028	0.028	0.009	0.012	0.005	0.019	---	0.012	0.025	0.010	0.029	0.018	0.019	0.006
119	0.028	0.007	0.056	0.008	0.011	0.028	0.027	0.066	0.031	0.042	0.058	0.044	0.035	0.051
121	0.488	0.500	0.426	0.512	0.526	0.602	0.345	0.398	0.327	0.333	0.362	0.364	0.323	0.308
123	0.260	0.254	0.259	0.303	0.323	0.204	0.482	0.416	0.451	0.453	0.406	0.382	0.494	0.468
125	0.008	---	---	0.020	0.013	---	0.009	0.012	0.006	0.016	0.007	0.022	0.013	0.032
Oki11														
78	0.133	0.121	0.100	0.030	0.035	0.047	0.100	0.152	0.125	0.104	0.112	0.091	0.097	0.108
84	0.558	0.557	0.630	0.607	0.654	0.651	0.418	0.518	0.425	0.448	0.485	0.530	0.526	0.519
86	0.304	0.321	0.270	0.363	0.311	0.302	0.473	0.305	0.431	0.432	0.388	0.370	0.367	0.354
88	0.004	---	---	---	---	---	---	---	---	---	---	---	---	---
92	---	---	---	---	---	---	---	0.006	---	---	---	---	---	---
114	---	---	---	---	---	---	0.009	0.018	0.019	0.016	0.015	0.009	0.010	0.019

Appendix 1 cont.

Locus Allele	<u>Gisasa R.</u>			<u>Tozitna R.</u>			<u>Tuluksak R.</u>				<u>Kwethluk R.</u>			
	1995	1996	1997	1997	1998	1999	1996	1997	1998	1989	1996	1997	1998	2002
Oke4														
238	---	---	---	---	---	---	---	---	---	---	---	---	---	0.007
240	0.226	0.331	0.314	0.257	0.270	0.202	0.200	0.218	0.197	0.241	0.210	0.248	0.219	0.297
243	0.668	0.600	0.667	0.540	0.533	0.644	0.733	0.662	0.659	0.618	0.637	0.593	0.657	0.616
246	0.106	0.069	0.020	0.204	0.197	0.154	0.067	0.120	0.144	0.141	0.153	0.159	0.124	0.080
Oki10														
100	---	---	---	---	---	---	---	---	---	---	---	---	---	0.007
128	---	---	---	---	---	---	---	---	---	---	---	---	---	0.007
156	---	---	---	---	---	---	---	---	---	---	---	0.004	---	0.013
160	---	---	---	---	---	---	---	0.006	---	---	---	0.004	---	---
164	---	---	0.009	---	---	---	---	---	---	---	---	---	---	---
168	0.008	0.015	0.009	---	---	---	0.020	---	0.006	0.005	0.007	---	0.010	0.007
172	0.008	---	0.018	---	0.003	0.009	0.020	---	---	0.027	0.015	0.004	0.020	0.007
176	0.042	0.022	0.027	---	---	0.009	0.020	0.038	0.070	0.033	0.015	0.009	0.027	0.013
180	---	---	---	---	0.008	0.019	0.020	0.006	0.013	0.016	0.007	0.013	0.013	0.007
184	0.008	0.037	---	0.009	0.014	0.019	0.069	0.013	---	0.016	0.015	0.009	0.030	0.040
188	0.004	0.022	0.027	0.030	0.038	0.066	---	0.019	0.025	0.016	0.022	0.022	0.017	0.020
192	0.008	0.007	0.009	0.004	---	0.009	0.039	0.006	0.006	0.022	0.015	0.013	0.017	0.027
196	---	---	0.009	0.026	---	---	0.029	0.032	0.019	0.011	0.015	0.035	0.027	0.027
200	0.017	0.007	0.009	0.091	0.036	0.028	0.010	0.026	0.032	0.016	0.015	0.017	0.027	0.027
204	0.017	0.030	0.027	0.013	0.008	0.019	0.010	0.026	0.019	---	0.051	0.030	0.020	0.033
208	0.013	---	0.009	---	0.003	0.009	0.029	0.026	0.025	0.033	0.029	0.009	0.033	0.013
212	0.034	0.022	0.036	0.056	0.049	0.057	0.029	0.051	0.019	0.049	0.022	0.048	0.043	0.027
216	0.055	0.060	0.064	0.073	0.074	0.038	0.078	0.026	0.019	0.049	0.037	0.039	0.057	0.040
220	0.029	0.052	0.027	0.022	0.011	0.019	0.059	0.045	0.032	0.049	0.044	0.061	0.040	0.053
224	0.046	0.060	0.073	0.052	0.060	0.047	0.049	0.058	0.032	0.044	0.037	0.035	0.043	0.013
228	0.080	0.075	0.100	0.047	0.080	0.075	0.029	0.083	0.051	0.038	0.066	0.035	0.040	0.033
232	0.021	0.022	---	0.043	0.052	0.019	0.029	0.032	0.057	0.044	0.037	0.035	0.033	0.040
236	0.029	0.037	0.009	0.013	0.025	0.047	0.020	0.019	0.051	0.011	0.051	0.026	0.033	0.040
240	0.017	0.022	0.018	0.013	0.044	0.019	---	0.013	0.025	0.033	0.022	0.022	0.023	0.020
244	0.004	0.022	---	0.004	0.008	0.028	---	0.013	0.013	---	0.022	0.009	0.010	0.007
248	---	---	0.009	0.004	0.005	---	0.010	0.026	0.006	0.005	---	---	0.013	---
252	0.008	0.007	0.009	0.004	0.003	---	0.010	0.006	---	---	---	0.013	0.007	---
256	---	---	---	---	0.003	---	---	---	---	0.005	0.007	0.013	0.003	---
260	---	---	---	---	---	---	---	---	0.006	0.033	0.007	---	0.007	0.013
264	---	0.015	---	---	---	---	---	---	---	0.005	0.015	0.009	---	0.007
268	---	---	---	---	0.003	---	---	---	---	0.005	---	---	---	---
272	---	---	---	---	---	---	---	0.013	---	---	---	0.013	0.007	0.007
276	0.004	---	0.027	---	---	---	0.010	0.019	0.038	0.005	0.015	0.030	0.013	0.033
280	0.034	0.037	0.027	0.004	0.005	---	0.020	0.013	0.013	0.033	0.015	0.026	0.027	0.020
284	0.088	0.075	0.045	0.017	0.041	0.038	0.029	0.038	0.044	0.022	0.074	0.052	0.040	0.033
288	0.021	0.022	---	0.065	0.047	0.028	0.069	0.026	0.032	0.038	0.059	0.039	0.040	0.033
292	0.021	0.022	0.036	0.047	0.082	0.047	0.049	0.045	0.032	0.027	0.044	0.048	0.057	0.080
296	0.092	0.060	0.082	0.030	0.038	0.057	0.029	0.045	0.063	0.055	0.044	0.065	0.047	0.007
300	0.113	0.060	0.036	0.091	0.077	0.028	0.069	0.058	0.063	0.071	0.059	0.048	0.043	0.047

Appendix 1 cont.

Locus allele	Gisasa R.			Tozitna R.			Tuluksak R.			Kwethluk R.				
	1995	1996	1997	1997	1998	1999	1996	1997	1998	1989	1996	1997	1998	2002
304	0.067	0.045	0.064	0.060	0.030	0.038	0.049	0.045	0.063	0.060	0.037	0.039	0.030	0.053
308	0.046	0.075	0.091	0.009	0.033	0.057	0.029	0.038	0.032	0.055	0.037	0.048	0.047	0.053
312	0.025	0.037	0.009	0.026	0.022	0.047	0.020	0.026	0.032	0.022	0.015	0.026	0.023	0.047
316	0.004	---	0.009	0.034	0.016	0.038	0.020	0.026	0.019	0.016	0.015	0.009	0.007	0.013
320	---	---	---	0.099	0.069	0.057	0.010	0.026	0.025	0.011	0.007	0.013	0.013	0.013
324	0.017	0.030	0.045	0.009	0.008	0.028	0.010	0.013	0.019	0.005	0.007	0.013	0.010	0.020
328	0.013	---	0.018	0.004	0.003	---	0.010	---	---	0.005	---	0.009	---	---
332	0.004	---	---	---	---	---	---	---	---	---	---	0.004	0.003	---
336	---	---	---	---	---	---	---	---	---	---	---	0.004	---	---
352	---	---	0.009	---	---	---	---	---	---	---	---	---	---	---

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202	0.010	0.008	0.010	0.021	0.011	---	---	---	---	---	---	0.009	0.007	0.006
258	---	---	---	---	---	---	---	---	---	---	---	0.009	---	---
262	---	0.008	---	---	---	---	---	---	---	---	---	---	---	---
266	---	---	---	---	---	---	---	0.006	0.007	---	---	---	---	---
270	0.019	0.023	0.010	0.009	0.014	0.020	0.037	---	0.013	0.011	---	0.005	0.004	---
274	0.038	0.030	0.010	---	---	---	---	0.006	0.013	0.011	0.008	---	0.007	---
278	0.014	0.008	0.010	0.009	0.020	---	0.037	0.018	---	0.005	0.023	0.014	0.033	0.032
282	0.057	0.045	0.030	0.009	0.003	---	0.009	0.018	0.046	0.016	0.046	0.042	0.022	0.006
286	0.005	0.008	0.010	0.034	0.020	0.020	---	0.006	0.013	0.016	0.008	0.023	0.029	0.026
290	0.038	0.030	0.040	0.021	0.017	0.020	0.037	0.024	---	0.005	0.008	0.009	0.018	0.013
294	0.010	0.023	0.050	0.017	0.023	0.010	0.019	0.037	0.026	0.032	0.015	0.028	0.018	0.019
298	0.024	0.053	0.050	0.013	0.037	0.020	0.009	0.012	0.020	0.021	0.023	0.033	0.044	0.038
302	0.133	0.038	0.110	0.021	0.026	---	0.028	0.012	0.039	0.026	0.023	0.037	0.059	0.051
306	0.100	0.076	0.080	0.068	0.060	0.092	0.083	0.079	0.039	0.074	0.054	0.070	0.063	0.051
310	0.076	0.098	0.080	0.077	0.074	0.051	0.065	0.067	0.059	0.068	0.038	0.084	0.051	0.096
314	0.071	0.091	0.070	0.081	0.057	0.020	0.074	0.061	0.039	0.053	0.054	0.070	0.063	0.032
318	0.052	0.144	0.040	0.056	0.043	0.041	0.037	0.055	0.053	0.032	0.046	0.075	0.051	0.038
322	0.024	0.023	0.030	0.051	0.034	0.071	0.056	0.110	0.039	0.058	0.069	0.047	0.040	0.071
326	0.019	0.038	0.020	0.098	0.114	0.133	0.056	0.037	0.072	0.084	0.100	0.019	0.074	0.045
330	0.052	0.023	0.020	0.026	0.023	0.061	0.037	0.043	0.105	0.058	0.046	0.065	0.055	0.051
334	0.038	0.038	0.040	0.030	0.029	0.010	0.046	0.085	0.079	0.089	0.031	0.047	0.048	0.064
338	0.043	0.038	0.040	0.094	0.069	0.092	0.056	0.049	0.033	0.047	0.062	0.023	0.040	0.058
342	0.029	0.061	0.060	0.026	0.009	---	0.019	0.049	0.039	0.068	0.031	0.047	0.040	0.083
346	0.038	0.023	0.030	0.017	0.023	0.020	0.074	0.037	0.033	0.026	0.054	0.061	0.033	0.013
350	0.029	---	0.040	0.056	0.117	0.173	0.037	0.049	0.066	0.026	0.062	0.033	0.033	0.032
354	0.024	0.008	0.010	0.077	0.069	0.061	0.046	0.043	0.053	0.047	0.031	0.028	0.063	0.026
358	0.014	0.008	0.010	0.017	0.031	0.031	0.028	0.018	0.066	0.058	0.038	0.019	0.033	0.032
362	0.014	0.015	0.050	0.013	0.011	0.041	0.056	0.037	0.007	0.016	0.023	0.037	0.022	0.038
366	---	0.015	0.010	0.004	0.017	---	0.019	0.006	0.007	0.026	0.031	0.005	0.022	0.032
370	0.019	0.008	0.010	0.004	0.003	---	---	0.012	---	0.005	0.031	0.019	---	0.006
374	0.010	0.023	0.010	0.017	0.003	0.010	0.037	0.018	0.007	0.005	0.023	0.019	---	0.006
378	---	---	---	---	0.006	---	---	---	0.013	---	0.008	0.009	0.007	---
382	---	---	0.010	---	---	---	---	---	---	---	---	---	0.004	0.013
386	---	---	---	---	0.003	---	---	---	---	0.011	0.008	0.009	---	---
390	---	---	0.010	0.034	0.034	---	---	---	0.007	0.005	---	0.005	0.015	---
394	---	---	---	---	---	---	---	---	---	---	---	---	---	0.019

Appendix 1 cont.

Locus allele	<u>Gisasa R.</u>			<u>Tozitna R.</u>			<u>Tuluksak R.</u>			<u>Kwethluk R.</u>				
	1995	1996	1997	1997	1998	1999	1996	1997	1998	1989	1996	1997	1998	2002
398	---	---	---	---	---	---	---	0.006	---	---	---	---	---	---
402	---	---	---	---	---	---	---	---	---	---	0.008	---	---	---
410	---	---	---	---	---	---	---	---	0.007	---	---	---	---	---
OtsG474														
152	---	---	---	---	---	---	---	---	---	---	---	---	---	0.026
156	0.976	0.931	0.973	0.923	0.953	0.926	0.963	0.975	0.966	0.989	0.949	0.948	0.964	0.942
160	---	---	---	---	---	---	---	---	0.007	0.006	---	0.017	---	---
164	0.004	0.028	0.018	0.077	0.045	0.065	0.019	0.006	0.007	---	0.036	0.017	0.023	0.019
168	---	---	---	---	---	---	---	---	---	---	---	0.013	0.003	0.006
176	0.008	0.014	0.009	---	---	---	0.009	0.013	0.021	---	0.014	---	0.006	0.006
180	0.008	0.014	---	---	0.003	0.009	0.009	0.006	---	0.006	---	0.004	0.003	---
184	0.004	0.014	---	---	---	---	---	---	---	---	---	---	---	---
OtsG432														
105	0.574	0.507	0.481	0.517	0.552	0.469	0.548	0.506	0.540	0.578	0.538	0.562	0.588	0.558
109	0.004	---	---	0.017	0.035	0.082	---	0.037	0.047	0.017	0.015	0.004	0.024	---
113	0.004	---	---	0.004	0.003	---	0.029	---	---	---	---	0.004	0.010	0.006
117	---	---	---	---	---	---	---	---	---	---	---	0.004	---	---
121	---	---	---	0.012	0.019	0.010	---	---	---	---	---	---	---	---
125	---	---	---	---	---	---	---	---	---	0.006	---	---	0.003	---
129	0.131	0.134	0.183	0.132	0.128	0.143	0.212	0.216	0.147	0.172	0.205	0.190	0.156	0.173
133	0.053	0.042	0.048	0.054	0.030	0.031	0.029	0.056	0.040	0.039	0.068	0.040	0.041	0.045
137	0.025	0.028	0.038	0.054	0.052	0.031	0.010	0.012	0.013	0.033	0.015	0.009	0.010	0.006
141	0.016	0.028	0.038	0.021	0.022	0.020	0.010	0.006	0.013	0.011	0.015	0.013	0.003	---
145	0.016	0.042	0.010	0.004	0.003	---	---	---	---	0.011	0.015	0.013	0.007	0.013
149	0.020	0.014	---	0.004	---	---	---	0.019	0.013	0.006	0.008	0.013	0.024	0.006
153	---	---	---	---	0.003	---	---	0.006	0.033	0.022	0.023	0.009	0.017	0.006
157	0.025	0.028	0.048	0.008	0.005	---	0.019	---	---	---	---	0.027	0.007	---
161	0.041	0.042	0.048	0.066	0.038	0.092	0.029	0.006	0.013	0.022	0.030	0.013	0.010	0.019
165	0.029	0.042	0.029	0.017	0.022	0.010	0.019	0.025	0.040	0.017	0.015	0.031	0.024	0.013
169	---	0.007	0.010	0.041	0.035	0.071	0.010	0.006	0.007	---	0.015	0.004	0.010	0.019
173	0.016	0.028	0.010	0.012	0.016	0.020	0.010	0.012	0.027	0.033	0.015	0.013	0.017	0.032
177	0.012	0.035	0.038	0.017	0.011	---	0.010	0.031	0.013	---	0.008	0.022	0.010	0.032
181	0.016	---	0.010	0.008	0.008	---	0.010	0.037	0.020	0.011	0.008	0.013	0.010	0.032
185	0.008	0.007	0.010	0.012	0.005	0.020	---	0.019	---	0.006	0.008	0.009	0.010	0.013
189	---	---	---	---	---	---	0.019	0.006	0.007	---	---	---	0.003	0.019
193	0.008	0.014	---	---	0.014	---	0.010	---	---	0.006	---	0.004	0.010	---
197	---	---	---	---	---	---	---	---	0.020	0.006	---	---	---	0.006
201	---	---	---	---	---	---	0.029	---	0.007	---	---	---	0.003	---
209	---	---	---	---	---	---	---	---	---	0.006	---	---	---	---
OtsG3														
133	---	---	---	---	---	---	---	---	---	---	---	---	---	0.014
135	0.355	0.264	0.327	0.443	0.386	0.435	0.330	0.360	0.365	0.345	0.425	0.449	0.402	0.318
137	0.448	0.521	0.418	0.415	0.455	0.417	0.351	0.400	0.397	0.458	0.358	0.346	0.331	0.385
141	0.198	0.214	0.255	0.142	0.159	0.148	0.319	0.240	0.237	0.196	0.216	0.206	0.267	0.284

Appendix 1 cont.

Locus allele	<u>Gisasa R.</u>			<u>Tozitna R.</u>			<u>Tuluksak R.</u>			<u>Kwethluk R.</u>				
	1995	1996	1997	1997	1998	1999	1996	1997	1998	1989	1996	1997	1998	2002
OtsG253														
120	---	---	---	---	---	---	---	0.006	---	---	---	---	---	---
124	---	---	---	---	---	---	0.009	0.006	0.006	0.005	0.029	0.013	0.007	0.006
128	0.062	0.029	0.009	0.013	0.014	---	0.009	0.025	0.006	0.016	0.014	0.004	0.003	0.006
132	0.012	0.007	0.028	0.013	0.025	---	---	0.006	0.019	0.011	0.022	0.009	0.013	---
136	0.012	---	0.019	0.017	0.016	0.020	0.019	0.038	0.050	0.033	0.022	0.040	0.040	0.019
140	0.116	0.157	0.075	0.102	0.107	0.137	0.074	0.081	0.050	0.114	0.109	0.121	0.107	0.083
144	0.037	0.079	0.028	0.051	0.047	0.029	0.019	0.038	0.031	0.033	0.051	0.049	0.053	0.058
148	0.021	0.050	0.028	0.004	0.005	0.010	0.065	0.063	0.063	0.049	0.014	0.049	0.050	0.038
152	0.050	0.071	0.104	0.097	0.077	0.049	0.037	0.044	0.081	0.054	0.058	0.076	0.083	0.051
156	0.058	0.079	0.028	0.161	0.088	0.078	0.065	0.063	0.056	0.038	0.072	0.036	0.047	0.103
160	0.095	0.071	0.085	0.076	0.102	0.176	0.093	0.063	0.069	0.038	0.101	0.089	0.083	0.045
164	0.041	0.036	0.094	0.017	0.014	0.029	0.046	0.031	0.038	0.054	0.116	0.107	0.073	0.096
168	0.079	0.064	0.075	0.034	0.066	0.069	0.028	0.031	0.088	0.082	0.043	0.040	0.027	0.032
172	0.037	0.043	0.028	0.042	0.044	0.020	0.056	0.038	0.063	0.054	0.036	0.022	0.060	0.051
176	0.095	0.014	0.057	0.081	0.066	0.098	0.037	0.050	0.006	0.054	0.051	0.027	0.050	0.045
180	0.033	0.043	0.047	0.068	0.088	0.098	0.065	0.075	0.056	0.033	0.043	0.049	0.050	0.077
184	0.062	0.057	0.038	0.047	0.041	0.059	0.065	0.056	0.088	0.043	0.043	0.054	0.040	0.058
188	0.045	0.057	0.028	0.064	0.069	0.078	0.083	0.094	0.075	0.049	0.043	0.063	0.040	0.026
192	0.033	0.036	0.075	0.013	0.041	0.020	0.093	0.063	0.056	0.082	0.036	0.045	0.050	0.058
196	0.004	0.014	---	0.021	---	---	0.028	0.038	0.025	0.027	0.007	0.018	0.013	0.038
200	0.012	0.007	---	0.004	0.011	---	---	0.025	0.019	0.016	0.022	0.022	0.027	0.019
204	0.079	0.050	0.104	0.008	0.025	0.010	0.028	0.025	0.013	0.038	0.022	0.027	0.020	0.026
208	---	---	0.009	---	---	---	0.056	0.038	0.044	0.022	0.014	0.013	0.023	0.006
212	---	---	---	---	---	---	---	---	---	0.016	0.007	0.013	0.027	0.013
216	0.004	0.021	0.038	0.004	0.008	0.010	---	---	---	0.022	0.014	0.004	---	0.013
224	---	---	---	---	---	---	---	0.006	---	---	---	---	---	---
244	---	---	---	---	---	---	---	---	---	---	---	---	---	0.006
248	---	---	---	0.013	0.003	---	---	---	---	---	---	---	---	0.006
252	---	---	---	0.004	---	---	---	---	---	---	---	---	0.003	---
256	---	---	---	---	---	---	0.009	---	---	---	---	---	---	---
260	---	---	---	---	---	---	---	---	---	---	---	---	0.003	---
264	---	---	---	---	0.005	---	0.009	---	---	---	---	---	---	0.006
268	---	---	---	0.004	0.003	---	0.009	---	---	---	---	---	0.007	---
272	---	---	---	---	0.005	0.010	---	---	---	---	---	---	---	---
276	---	---	---	0.004	---	---	---	---	---	---	---	---	---	---
280	0.004	---	---	0.021	0.019	---	---	---	---	---	---	---	---	---
284	0.008	0.014	---	0.017	0.011	---	---	---	---	0.016	0.007	0.009	---	0.006
288	---	---	---	---	---	---	---	---	---	---	---	---	---	0.006